Table 4. *The influence of sex on bone ash content*

Diet	Range of % ash in the humerus	No. of animals ♂	No. of animals ♀	Average value of d ♂	Average value of d ♀	Average value of $(d \times 100)/H$ ♂	Average value of $(d \times 100)/H$ ♀
Rachitogenic	24–27	18	9	6·29	6·07	23·4	22·4
	28–31	35	26	6·49	6·02	21·7	19·9
	32–35	20	15	5·89	5·60	17·4	16·4
	36–39	12	8	4·38	6·07	11·7	16·0
	40–43	10	12	3·16	3·77	7·6	9·1
	44–47	3	8	4·58	1·38	9·7	3·0
	48–51	7	8	1·09	1·09	2·2	2·2
Low Ca	36–39	10	5	0·12	−0·50	0·3	−1·4
	40–43	8	13	−0·43	−0·12	−1·1	−0·2
	44–47	20	12	0·38	−0·24	0·8	−0·5
	48–51	17	8	0·82	0·08	1·6	0·2
	52–55	6	10	1·34	1·55	2·5	2·9
Stock	54–62	20	18	0·17	0·05	0·3	0·1

at both 100 and 150 days of age the humerus and femur of female rats have a higher percentage of ash than those of male rats. The figures given in Table 1 have been analysed in Table 4 according to the sex of the animal. The differences in ash content between the rachitic humerus and femur is shown equally by both sexes.

SUMMARY

1. When low calcification in rats, as measured by the ash content of the humerus, is due to shortage of vitamin D, the femur shows an even lower calcification. When, however, it is due to a deficiency of either Ca or P without a simultaneous shortage of vitamin D in the diet, no difference in calcification between the two bones is observed, neither is a difference found in the bones of rats receiving a stock diet.

2. In the femur both the relatively large metaphysis and the extremely low calcification of this part make the ash content of the whole bone lower than that of the humerus when there is a deficiency of vitamin D in the diet.

3. The differences between the rachitic humerus and femur are not influenced by the sex of the animal.

The authors wish to express their thanks to Prof. J. H. Gaddum and Dr K. H. Coward for their help and interest throughout the course of this work.

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The Preparation of Deoxycholic Acid from Cholic Acid

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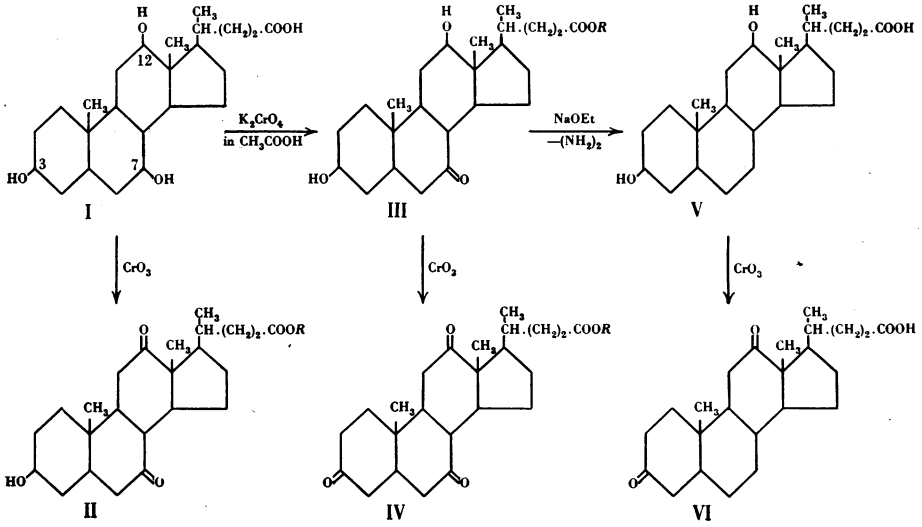
(*Received 21 October 1942*)

Deoxycholic acid (3:12-dihydroxycholanic acid) has been made from cholic acid in small yield by hydrogenation of the derived 3:12-dihydroxycholenic acid [Boedecker & Volk, 1922], but this method is of academic interest only. The present work, as already stated [Haslewood, 1942], aims at the production of deoxycholic acid by a convenient method from the readily available cholic acid, to supplement the

rather uncertain preparation from bile of Na deoxycholate for bacteriological use. Kaziro & Shimada [1937] showed that cold chromic oxidation of cholic acid (I) gave 3-hydroxy-7:12-diketocholanic acid (II, $R=H$): this was confirmed by Bergström & Haslewood [1939]. Moreover, although Kaziro & Shimada could not obtain a mono-ketonic acid, they indicated that such a substance might be formed, in an overall yield of about 30% of theory. Deoxycholic acid, also of good quality and apparently free from cholic acid, was given in an overall yield of 40–50% of theory by direct reduction of the crude oxidation product. Lithocholic acid may have been an impurity in this final material, for, although it was not apparent therein, it was found in small amount in the mother-liquors. Reduction



since Wieland & Dane [1932] had shown that 7:12-dihydroxycholanic acid was oxidizable to give a 7-keto-12-hydroxy derivative. Direct chromic oxidation of cholic acid has now been made to yield the expected derivatives of 7-keto-3:12-dihydroxycholanic acid (III), easily reduced to deoxycholic acid (V). This, isolated as the ether-complex, was identified by its usual properties and by conversion into the characteristic dehydrodeoxycholic acid (VI).

Controlled chromic oxidation was carried out by the addition of K_2CrO_4 to cholic acid dissolved in acetic acid. Many experiments showed that the highest yield of 7-keto-compounds was obtained (*a*) when the solvent was highly purified acetic acid, and (*b*) when the reaction was greatly slowed. These conditions were met by the addition of measured amounts of K_2CrO_4 in concentrated solution and by buffering the mixture with Na acetate. This method enabled a crystalline semicarbazone (mainly of 7-keto-3:12-dihydroxycholanic acid III, $R=H$) to be isolated in yields of about 45% of theory when chromate was added in amount equivalent to one atom of oxygen. The semicarbazone, however, contained small amounts of cholic acid, which appeared in the final product. This impurity could be removed, but better yields were obtained by using chromate equivalent to two atoms of oxygen, when the semicarbazone, though contaminated with high-melting material, gave a very pure deoxycholic acid [cf. Longwell & Wintersteiner, 1940; Kon & Soper, 1940] was performed with Na ethylate-hydrazine at 200° for 3 hr., with the use of minimal amounts of Na.

The intermediates (III), 7-keto-3:12-dihydroxycholanic acid and its ethyl ester, were obtained from the oxidation product or from the semicarbazone. On acid-alcoholic hydrolysis, the latter gave *ethyl 7-keto-3:12-dihydroxycholanate* (III, $R=C_2H_5$), M.P. (uncorr.) 160–161°, depressed by ethyl 3-hydroxy-7:12-diketocholanate (II, $R=C_2H_5$) but not by ethyl cholate. The same ester M.P. 160–161° was isolated after esterification of the crude chromic oxidation product. It is easier to prepare the 7-keto-ester (the most easily purified of the intermediates) by over-, rather than by under-oxidation of cholic acid, since its separation from ethyl cholate is difficult. The crystalline ester (III, $R=C_2H_5$) was oxidized to ethyl dehydrocholate (IV, $R=C_2H_5$) and reduced, with saponification, to deoxycholic acid. The interesting *7-keto-3:12-dihydroxycholanic acid* (III, $R=H$), from the ethyl ester, separated from dilute aqueous acid as hydrated crystals, M.P. *ca.* 83°, and could be recrystallized from the same solvent although it did not crystallize from organic solvents in the usual way. The cholic acid derivatives could be distinguished from the others here mentioned by the HCl test of Hammarsten [1909], which gave a yellow colour with the 7-keto-compounds (III) and

green colours with mixtures of these and substances with the cholic acid nucleus. The test was of great value during this investigation.

EXPERIMENTAL

All M.P. are uncorrected. Elementary micro-analysis was carried out by Dr H. Nisbet, Edinburgh. Deoxycholic acid was obtained as the ether-complex, M.P. 150–155°. Samples of every preparation obtained in altered conditions were oxidized in the usual way with CrO_3 to give dehydrodeoxycholic acid, crystallizing from dilute alcohol in characteristic glistening leaflets of M.P. 184–186°, decomp., not depressed by authentic material.

Hammarsten's HCl test was done by boiling a mixture of 2–3 mg. of material with 0·2 ml. of strong HCl in a small tube for 1–2 min. The characteristic colours appeared after 1–2 hr.

Preparation of deoxycholic acid. (*a*) *Oxidation and purification.* *n* g. ($n = 1$–10) of dried and powdered cholic acid were dissolved at 60–70°, in a flask of at least 100*n* ml. capacity, in 10*n* ml. of glacial acetic acid. At the same temperature, 2*n* g. of $CH_3COONa.3H_2O$ crystals were added and dissolved. The mixture was cooled at once to 18–20° and treated with 2*n* ml. ($\equiv 2n$ atoms of O) of K_2CrO_4 solution (31·7 g. of K_2CrO_4/100 ml.), added slowly with shaking, agitation at intervals being continued until all precipitated chromate had dissolved. After at least 24 hr., the solution was diluted with 70*n* ml. of H_2O, and 10*n* g. of crude NaCl were added. When this had dissolved, and after further standing, with occasional shaking, for 24 hr., the liquor was decanted through a filter and the residue and filter washed with H_2O. 10*n* ml. of *N* NaOH followed by 20*n* ml. of H_2O were poured through the filter and back into the original flask. This was now heated, with agitation, until the contents dissolved and the solution gently boiled. After 5–10 min. boiling and when Cr compounds had coagulated, the flask was partially cooled and the still warm mixture treated with 5*n* g. of crude NaCl, dissolved with shaking, followed by 20*n* ml. of *N* H_2SO_4. The precipitate was collected after 16 hr. and well washed on the filter with cold H_2O. It was then allowed to become partially dry, and dissolved in cold alcohol. The filtered solution was evaporated, finally at the pump, leaving 0·8–0·9*n* g. of a yellowish gum (*product A*) giving a yellow colour in the Hammarsten test.

(*b*) *Reduction.* 5 ml. of an ethyl alcoholic solution containing 40 g./100 ml. of *product A* were added to a metal bomb containing a solution of 0·4 g. Na in 5 ml. ethyl alcohol, with 2 ml. of hydrazine hydrate (95/98%). The mixture was carefully heated almost to boiling; then the bomb was sealed and heated at 200–210° for 3 hr. An aqueous extract (*ca.* 200 ml.) of the contents of the cooled bomb was acidified with dilute H_2SO_4, the precipitate being warmed gently to disperse gels. After 2–3 hr., the white solid was collected, washed with water and dissolved in cold alcohol. Evaporation of the filtered solution left a gum which crystallized at once when about 10 ml. of ether were added. After warming with the ether, the product was allowed to stand for several hours with occasional shaking, and was finally cooled to 0°. The crystals were collected (suction), washed with ice-cold ether, and dried. Yield, 1·1–1·2 g.; small white needles M.P. 150–155°, HCl-test, faint yellow-green. 0·2 g. of this substance, from 2–3 ml. of acetic acid, gave 0·17 g. of white needles M.P. 139–141°.

4 g. of residue obtained on evaporation of the ether mother liquors from (*b*) were dissolved in a little fresh ether. During 24 hr., crystals accumulated. The liquor was decanted and the crystals washed with ether and cold alcohol, collected, and dried. This material (0·15 g.) had M.P. 184–186°, not depressed by lithocholic acid; it was not obviously affected by HCl.

Semicarbazone. To *product A*, from *n* g. of cholic acid, were added *n*/2 g. of semicarbazide hydrochloride, *n*/2 g. of Na acetate crystals, and 5*n* ml. of 50% (by vol.) alcohol/water. This mixture was warmed till all had dissolved, boiled under reflux for 1 hr. and allowed to stand 3–5 days. The solid was collected (suction), washed with 50% alcohol, water, alcohol, and ether, and dried at 60–80°. Yield, 0·6*n* g., M.P. >330°; HCl-test, yellow. (Found, N, 11·2%.) When the oxidation (*a*) was done with 1·1*n* ml. of K_2CrO_4 solution ($\equiv 1{\cdot}1n$ atoms of O), the semicarbazone (0·5*n* g.), prepared as described, formed groups of white needles, M.P. 250° (decomp.), which gave a blue HCl-test as also did the deoxycholic acid and 7-keto-derivatives from them. (Found, N, 8·6%.) 0·5 g. of this latter product, after boiling with 5 ml. of alcohol under reflux for 30 min. left 0·32 g. of undissolved crystals, M.P. 265°, decomp.; HCl-test, yellow-green. (Found, N, 10·0%. $C_{25}H_{41}O_5N_3$ requires N, 9·1%.) A product of M.P. 267°, decomp.; HCl-test, green, was given when $K_2CrO_4 \equiv 1{\cdot}5n$ atoms of O was used for the oxidation (*a*).

The above semicarbazones could be reduced by heating (in a sealed bomb at 200–210° for 3 hr.) 1 g. samples with a solution of 0·25 g. of Na in 10 ml. ethyl alcohol, with 1 ml. of hydrazine hydrate. The deoxycholic acid, obtained as under (*b*), had similar properties, that from the semicarbazone M.P. >330° appearing to be of especially good quality. Overall yield of deoxycholic acid via the semicarbazone, about 30% of theory.

Ethyl 7-keto-3:12-dihydroxycholanate. (i) 6·5 g. of *product A* were boiled for 45 min. under reflux with 13 ml. of a mixture of ethyl alcohol (20 ml.) and concentrated H_2SO_4 (5 ml.). The cooled solution was diluted with water and extracted three times with ether. The ether, washed with water and Na_2CO_3 solution, was evaporated, and the residue crystallized from methyl alcohol; 1·6 g. of crude crystals were obtained.

(ii) 3 g. of semicarbazone M.P. >330° were boiled under reflux for 1 hr. with 30 ml. of a mixture of ethyl alcohol (40 ml.), H_2O (10 ml.), and conc. H_2SO_4 (5 ml.). From the cooled and diluted solution, crude crystals (0·9 g.) were obtained exactly as under (i). The ester crystallized from methyl alcohol in large colourless rectangular plates or prisms, which, after drying at 70–80°, had M.P. 160–161°, not depressed by ethyl cholate. (Found, C, 72·0; H, 9·9%. $C_{26}H_{42}O_5$ requires C, 71·9; H, 9·7%.) The compound formed glistening leaflets from a diluted methyl alcoholic solution: it gave a yellow colour with strong HCl.

A purified sample of ethyl 7-keto-3:12-dihydroxycholanate was oxidized in the usual way with CrO_3 to ethyl dehydrocholate, M.P. 222–223° (decomp.), and another sample was reduced as under 'Semicarbazone' to deoxycholic acid (0·13 g. from 0·2 g. of ester, M.P. 160–161°). By the same process, 0·2 g. of ethyl cholate gave 0·14 g. of cholic acid.

7-keto-3:12-dihydroxycholanic acid. 0·3 g. of the above ester, M.P. 160–161°, was boiled under reflux for 30 min. with a mixture of Na (70 mg.), ethyl alcohol (1·5 ml.), and H_2O (1 drop), the ester being added after the Na had all dissolved. After dilution the cold solution was filtered, further diluted to 200 ml., heated to boiling and acidified with

5 ml. of N H_2SO_4. After 3–4 days, the white needles which had formed were collected and dried *in vacuo*. Yield, 0·13 g., M.P. 83°, giving a yellow colour with concentrated HCl and in the Liebermann-Burchard test, and a red colour with concentrated H_2SO_4. There was difficulty in removing solvent from this substance for analysis. (Found: C, 70·8; H, 9·5%. M (titration), 395. $C_{24}H_{38}O_5$ requires C, 70·9; H, 9·4%; M, 406.) The acid could be recrystallized by acidification of a hot alkaline solution as described, but did not form crystals from organic solvents. It dissolved easily in alcohol, acetone, and acetic acid, and to some extent in ether, but appeared to be insoluble in benzene and light petroleum.

SUMMARY

1. By a simple three-stage process (oxidation, purification, reduction), cholic acid has been converted into deoxycholic acid, with an overall yield of 40–50% of theoretical.

2. Preferential oxidation of cholic acid at C_7 was performed by adding measured amounts of concentrated K_2CrO_4 solution to cholic acid dissolved in acetic acid buffered with Na acetate.

3. The partially purified product was then reduced with Na ethylate-hydrazine, yielding deoxycholic acid. Deoxycholic acid was also prepared from an impure semicarbazone, the preparation and properties of which were used to follow and define the conditions of the oxidation. The intermediate 7-keto-3:12-dihydroxycholanic acid and its ethyl ester have been isolated and examined.

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Dental Depigmentation in the Rat

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(*Received 9 November 1942*)

In normal rats the anterior surfaces of the incisor teeth are a rich brown or orange. According to Marshall [1921] the pigment is confined to the outer fibrous layer. The whitening of the teeth which occurs in vitamin A deficiency has been described by Wolbach & Howe [1925; 1933], Smith & Lantz [1933], Schour, Smith & Hoffman [1938], Schour, Hoffman & Smith [1941], Irving & Richards [1939] and H. Mellanby [1939]. In recent experiments on the interaction of vitamin A and vitamin E, Davies & Moore [1941] found that under certain circumstances whitening of the teeth may result from the exclusion of vitamin E from the diet. It was suggested that this lesion might be caused by deficiency of either vitamin. Evidence in favour of this view is given in the present communication.

EXPERIMENTAL

Grading of depigmentation. To assess the degree of depigmentation in individual rats each tooth was awarded marks according to the following arbitrary scale.

Description	Marks given
White	25
Faint brown mottling on white	20
Cream	15
Mottled brown and white	10
Deep cream	10
Light brown	5
Brown	0

Thus the dental depigmentation of a rat with four completely white teeth is expressed by 100 marks, while four completely brown teeth are indicated by 0 marks.

The cure of white teeth in rats deficient in vitamin A by the administration of carotene

In routine tests for vitamin A in samples of butter and margarine, male albino and piebald rats were given a basal diet of caseinogen (Glaxo, alcohol extracted) 20%, sugar 60%, arachis oil 15%, salt mixture 5% and dried yeast 10%. Vitamin E was supplied as 1 mg. weekly of *dl*-α-tocopherol acetate dissolved in arachis oil, and vitamin D as 1 drop of 'Radiostol' (B.D.H.). In addition small doses of